

What is claimed is:

1. A compound of Formula 1:

R-L-S

(I)

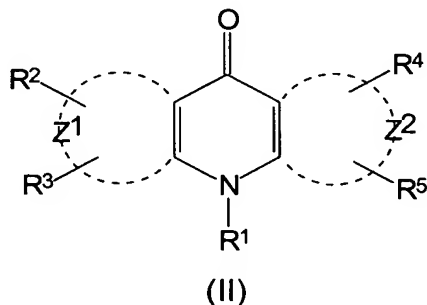
wherein R is a fluorescent dye molecule;

L is an optional linkage group containing one or more atoms comprising hydrocarbon chains which may also contain other atoms such as N, O and S; and

S is molecule comprising a substrate group of the enzyme aromatase

characterised in that the fluorescence signal of said compound changes in respect of fluorescence lifetime when the compound is acted upon by an enzyme with aromatase activity.

2. A compound according to claim 1 wherein said R is an acridone dye of Formula II:



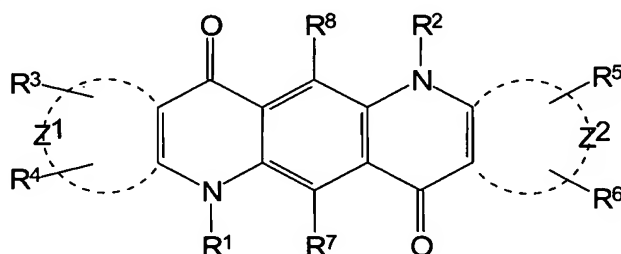
wherein:

groups R² and R³ are attached to the Z¹ ring structure and groups R⁴ and R⁵ are attached to the Z² ring structure;

Z¹ and Z² independently represent the atoms necessary to complete one or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;

R^1 , R^2 , R^3 , R^4 and R^5 are independently selected from hydrogen, halogen, amide, hydroxyl, cyano, amino, mono- or di- C_1 - C_4 alkyl-substituted amino, sulphydryl, carbonyl, C_1 - C_6 alkoxy, aryl, heteroaryl, C_1 - C_{20} alkyl, aralkyl; the group -E-F where E is a spacer group having a chain from 1-60 atoms selected from the group consisting of carbon, nitrogen, oxygen, sulphur and phosphorus atoms and F is a target bonding group; and the group $-(CH_2)_nY$ where Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary ammonium and carboxyl and n is zero or an integer from 1 to 6.

3. A compound according to claim 1 wherein R is a quinacridone dye of Formula III:



(III)

wherein:

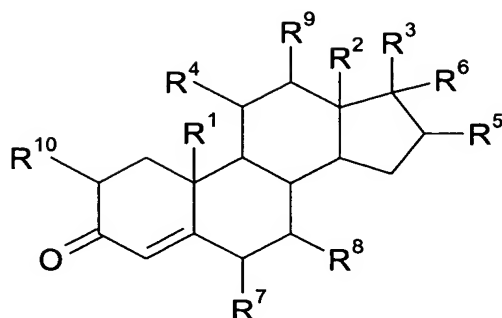
groups R^3 and R^4 are attached to the Z^1 ring structure and groups R^5 and R^6 are attached to the Z^2 ring structure;

Z^1 and Z^2 independently represent the atoms necessary to complete one or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;

R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 and R^8 are independently selected from hydrogen, halogen, amide, hydroxyl, cyano, amino, mono- or di- C_1 - C_4 alkyl-substituted amino, sulphydryl, carbonyl, carboxyl, C_1 - C_6 alkoxy, aryl, heteroaryl, C_1 - C_{20} alkyl, aralkyl; the group -E-F where E is a spacer group having a chain from 1-60 atoms selected from the group consisting of carbon, nitrogen, oxygen, sulphur and phosphorus atoms and F is a target bonding group; and the group $-(CH_2)_nY$ where Y is selected from sulphonate, sulphate, phosphonate, phosphate,

quaternary ammonium and carboxyl and n is zero or an integer from 1 to 6.

4. A compound according to any of claims 1 to 3 wherein L is a linker group containing from 1 to 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from $-NR'$ -, $-O$ -, $-S$ -, $-CH=CH$ -, $-C\equiv C$ -, $-CONH$ - and phenylenyl groups, wherein R' is selected from hydrogen and C_1 to C_4 alkyl.
5. A compound according to any of claims 1 to 4, wherein L is a linker group containing from 2 to 30 atoms.
6. A compound according to any of claim 1 to 5, wherein L is a linker group containing from 6 to 20 atoms.
7. A compound according to any of claims 1 to 6, wherein L is a linker group selected from the group: $\{(-CHR')_p-Q-(-CHR')_r\}_s$ where each Q is selected from CHR' , NR' , O , $-CH=CH$ -, Ar and $-CONH$ -;
- each R' is independently hydrogen or C_1 to C_4 alkyl;
each p is independently 0 to 5;
each r is independently 0 to 5;
and s is either 1 or 2.
8. A compound according to claim 7, wherein Q is selected from the group consisting of $-CHR'$ -, $-O$ - and $-CONH$ -, where R' is hydrogen or C_1 to C_4 alkyl.
9. A compound according to any preceding claim wherein S is a substrate group of the enzyme aromatase of formula IX



(IX)

wherein:

R^1 and R^2 are selected from H and methyl;

R^3 is selected from H, C_1 - C_8 alkyl, cyano, $-(CH_2)_k-OR^a$;

$-(CH_2)_k-COOR^a$; $-(CH_2)_k-SO_3R^a$; $-(CH_2)_k-CHO$, $-(CH_2)_k-NR^bR^c$ and $-(CH_2)_k-COR^d$;

R^4 is selected from H, $-COR^a$ and hydroxyl;

R^5 is selected from H, $-COR^a$, hydroxyl, cyano and halide;

R^6 is selected from H and hydroxyl;

R^7 , R^8 and R^9 are independently selected from H, $-COR^a$ and hydroxyl;

R^{10} is selected from H and halide; and

where R^a is selected from H and C_1 - C_4 alkyl, optionally substituted

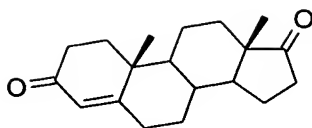
with OH; R^b and R^c are selected from H and C_1 - C_4 alkyl;

R^d is selected from C_1 - C_8 alkyl or C_1 - C_8 alkyl optionally substituted with $COOR^a$, OH, OR^a or SO_3R^a ;

and k is zero or an integer from 1 to 8.

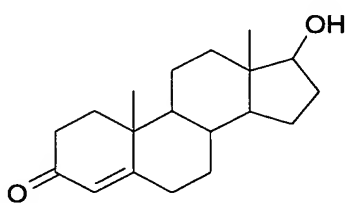
10. A compound according to claim 9 wherein Group S is a steroid selected from the group of steroid families consisting of 4-androsten-3-one, 4-cholesten-3-one, 4-estren-3-one and 4-pregnen-3-one derivatives.

11. A compound according to any of claims 1 to 10 wherein S is androstenedione of Formula X or a derivative thereof.



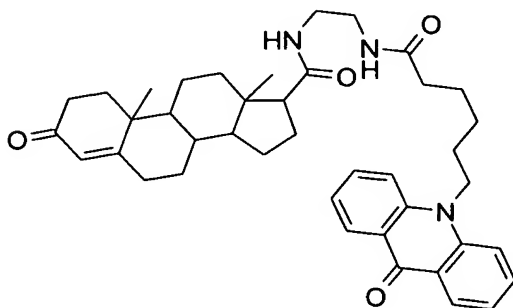
(X)

12. A compound according to any of claims 1 to 10 wherein S is testosterone of Formula XI or a derivative thereof.



(XI)

13. A compound according to any preceding claim of Formula XX



(XX)

14. A method for measuring aromatase activity in a sample, the method comprising the steps of:
- i) measuring the fluorescence lifetime of a compound according to any preceding claim prior to adding it to said sample;
 - 5 ii) adding said compound to said sample under conditions which favour aromatase activity, and
 - iii) measuring a change in fluorescence lifetime of said compound following step ii);
- wherein said change in fluorescence lifetime can be used to determine aromatase activity.
- 10
15. A method according to claim 14 wherein the sample is selected from the group consisting of extract, cell, tissue and organism.
- 15 16. A method of screening for a test agent whose effect upon the activity of aromatase is to be determined, said method comprising the steps of:
- i) performing the method of claim 14 or 15 in the presence of said agent; and
 - 20 ii) comparing the activity of said aromatase in the presence of the agent with a known value for the activity of aromatase in the absence of the agent;
- wherein a difference between the activity of the aromatase in the presence of the agent and said known value in the absence of the agent is indicative of the effect of the test agent upon the activity of aromatase.
- 25
17. The method according to claim 16, wherein the known value is stored upon an electronic database.
- 30 18. A method of screening for a test agent whose effect upon the activity of aromatase is to be determined, said method comprising the steps of:
- i) performing the method of claim 16 or 17 in the presence and in the absence of the agent; and

- ii) determining the activity of said enzyme in the presence and in the absence of the agent;

wherein a difference between the activity of aromatase in the presence and in the absence of the agent is indicative of the effect of the test agent upon the activity of aromatase.

19. The method according to claim 17 wherein said difference in activity between the activity of aromatase in the absence and in the presence of the agent is normalised, stored electronically and compared with a value of a reference compound.

20. A method for measuring the distribution of a compound of any of claims 1 to 13 within a tissue, wherein the compound is capable of being taken up by a living cell within said tissue, the method comprising the steps of:

- i) measuring the fluorescence lifetime of the compound in a cell-free environment or a parental host cell;
- ii) adding the compound to one or more cells or a cell engineered to over-express aromatase, and
- iii) measuring the fluorescence lifetime of the compound following step ii);

wherein a change in fluorescence lifetime indicates aromatase activity and can be used to determine the distribution of the compound.

21. A method according to claim 20, wherein the distribution of the compound within the tissue of a first subject is compared with the distribution of the compound within the tissue of a second subject.

22. The method of claim 21, wherein said subject is selected from the group consisting of mammal, plant, insect, fish, bird, fly, nematode and algae.

23. The method of claim 22, wherein the mammal is a mouse or a rat.

24. Use of a compound according to any of claims 1 to 13 for measuring aromatase activity as an *in vitro* or an *in vivo* imaging probe.
- 5 25. A method of diagnosing a disease caused by an increase in aromatase activity in a subject using the method according to claim 14, comprising comparing the activity of aromatase in a sample taken from a first subject with the activity in a sample taken from a second healthy control subject, wherein any increase in activity measured in the sample taken from the first subject relative to the second healthy control subject is indicative of disease.
- 10 26. Kit comprising:
- 15 i) a compound according to any of claims 1 to 13;
ii) an assay buffer; and optionally
iii) a stop buffer.